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Response of Cell Cultures to Asbestos Fibers

by J. Bruch*

The reaction of macrophages to asbestos and glass fibers was studied by light and by electron microscopy. Measurements were made of LDH release, staining with erythrosine B, and lactate production. Size and shape of asbestos fibers were found to have more effect than the physicochemical properties of the surface. Long fibers resulted in a protracted pathogenic but no acute cytotoxic effect.

I would like to present some results concerning the action of asbestos dust on different cells in vitro. These studies were carried out in our laboratory in collaboration with Prof. Beck and Dr. Manojlovic.

The main object of the investigations was to clarify whether asbestos fibers have a cytopathogenic effect and if so to compare this effect to that of other dusts. Established cell lines as well as macrophages were exposed to different types of asbestos and to glass fibers. In addition fibers of different lengths and milled fibers of glass and asbestos were used in order to elucidate the effect of morphology in the pathogenicity of asbestos.

The cells were studied by light microscopy and electron microscopy during successive phases of particle incorporation. Concomitantly biochemical studies were carried out. The functional state of the cell membrane as regards permeability was tested by determinations of lactate dehydrogenase (LDH) and cell staining with erythrosin B. Increased LDH release into the tissue culture supernatant is a very sensitive criterion for membrane leakage. The ability to stain with erythrosin B also indicates impairment of cellular membranes. Moreover, by this method the distinct condition of individual cells can be visualized by their staining behavior. Finally lactate production was measured as one parameter of glucose metabolism.

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Morphological studies by phase contrast and interference contrast microscopy show that many fibers are incorporated by all types of cells employed. Longer fibers are phagocytosed by several cells at the same time. The direct attachment of lysosomes to portions of extremely long fibers prove that these fibers are indeed localized intracellularly.

Electron microscopy clarified that all successive stages of phagocytosis can be present simultaneously along a single fiber. Figure 1 shows a longitudinally sectioned fiber partially situated extracellularly, in a phagosome, and intracellularly in the cytoplasm without membrane association.

Figure 2 demonstrates all the stages of the incorporation of smaller fibers of 1–3 μm length. It is remarkable that in this test system no acute necrobiotic effect or lysosomal damage could be observed with asbestos concentrations up to 100 $\mu g/10^6$ cells at any time, even after continuous cultivation of the exposed cells for several weeks.

Biochemically some specific reactions were found, which are summarized in Figures 3 and 4. Some of the metabolic changes are also produced by toxic dusts like quartz. The LDH level and the ratio of LDH stained cells are raised, indicating abnormal membrane permeability. On the other hand, glucose metabolism (lactate production) is elevated over controls, proving the continuing viability of the culture. A higher transitory lactate production is found in

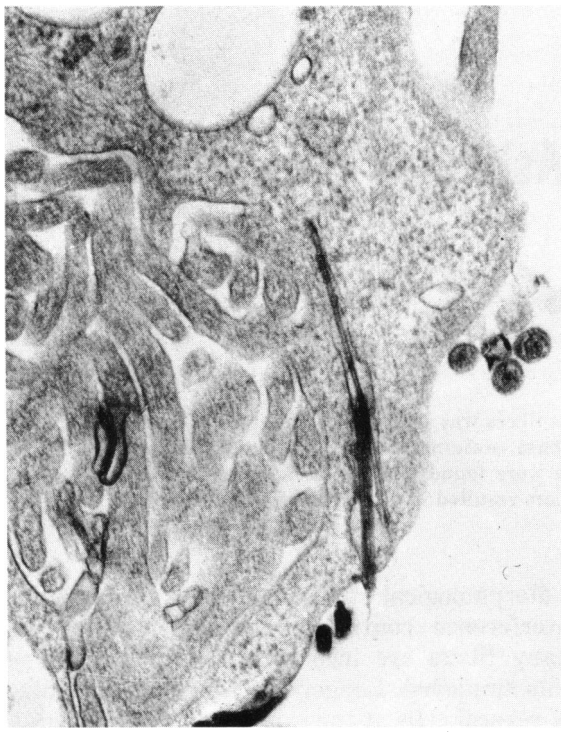

FIGURE 1. Alveolar macrophage with a chrysotile fiber situated partially extracellularly and partially intracellularly without membrane association. 49,000×

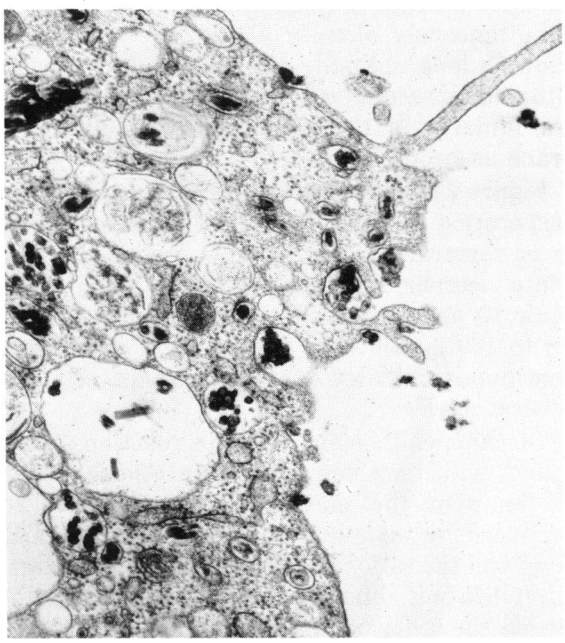

FIGURE 2. Phagocytosis of smaller chrysotile fibers with all successive stages of incorporation. 25,800×

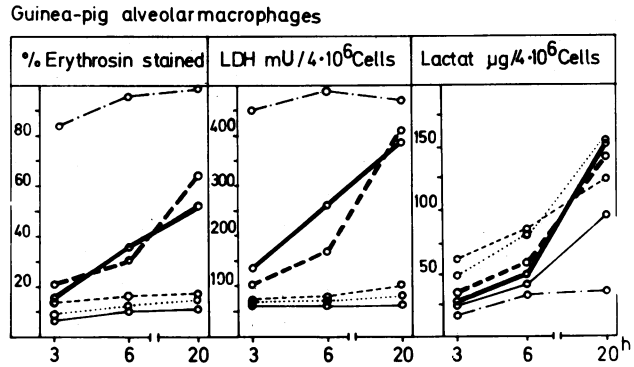

FIGURE 3. Guinea pig alveloar macrophages: (o——o) control; (o—·—o) quartz; (o . . . o) corundum; (o——o) chrysotile; (o--o) glass fiber; (o--o) glass powder. Concentration, 150 µg/10⁶ cells.

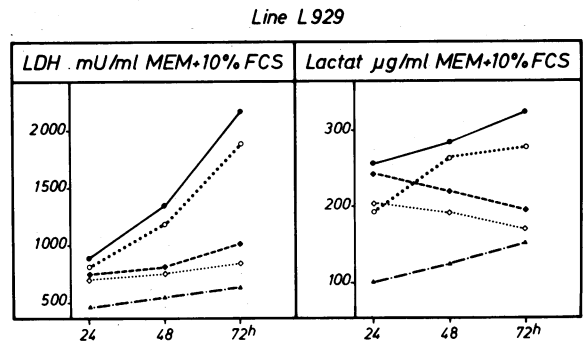

FIGURE 4. Biochemical indicators in the presence of various particles: (•——•) chrysotile, long; (•---•) chrysotile, milled; (o . . . o) glass fiber, long; (◊----◊) glass fiber, milled; (▲—·-▲) control. Concentration, 1 mg/10⁶ cells.

the beginning of the particle incorporation of all dusts expressing the active process of phagocytosis. In this case, however, the peculiar combination of high lactate production and elevated permeability of cell membrane (LDH and erythrosin test +++) after the initial phagocytic phase persists in established cell lines up to 3 months after a single exposure to long asbestos or glass fibers. On the other hand, milled asbestos or glass fibers display a morphological and metabolic pattern comparable to that with inert dusts like corundum.

These data indicate that the size and shape of the fibers are essential factors contributing to the pathogenicity of asbestos is and are more important than the physicochemical properties of the asbestos surface. Long fibers seem to produce a protracted pathogenic but no acute cytotoxic effect by the special defective phagocytosis on cellular level. *In vivo* experiments substantiate these *in vitro* studies.